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Operational sex ratio in terrestrial isopods: interaction between potential rate of reproduction and *Wolbachia*-induced sex ratio distortion

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Selfish genetic elements distorting sex ratio are known in several arthropods. By inducing a deficit of males, these sex ratio distorters may modify sexual selection by reversing the sex that competes for a mate. They also have potential to reduce the male proportion to values limiting mating possibilities and therefore limiting population size. *Wolbachia* endosymbionts are intracytoplasmic, vertically transmitted bacteria that convert genotypic males of terrestrial isopods (woodlice) into functional females. We have tested the impact of these feminizing symbionts on the operational sex ratio (OSR) in three woodlice species. Preliminary experiments consisted in estimating the potential rate of reproduction in males and females, and measuring the dynamics of the onset of reproduction in the wild. These parameters were then combined with population sex ratio to discriminate key factors influencing the OSR. The results suggest that the high potential rate of reproduction of males and the asynchrony in female receptivity both counterbalance female-biased sex ratios. The result is an overall balanced or slightly female-biased OSR. Male deficit can therefore not be considered as a factor strongly limiting reproduction in woodlice. Some females were nevertheless found not mated in the wild at the beginning of the reproductive season, most of them being infected by *Wolbachia*. This suggests that uninfected females may have an advantage as the first mate. Consequences of these findings on woodlice population dynamics are discussed.

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The concept of operational sex ratio (OSR, the ratio of fertilisable females to sexually active males at any given time) was first introduced by Emlen and Oring (1977) to summarise a number of important factors into one term to analyse mating systems and the pattern of sexual selection. The value of OSR can predict which sex will be in excess for reproduction, and hence both which sex will compete for access to mates, and also the intensity of this competition (Kvarnemo and Ahnesjö 1996). The parameters influencing the OSR are numerous, but most of them can be summarised by differences between sexes in their potential rate of reproduction (Clutton-Brock and Vincent 1991). Because of differences in paternal investment, males typically have the potential to reproduce faster and more often than females, i.e. the reproductive "time in" is higher in males than in females (Clutton-Brock and Parker 1992). The OSR is therefore male-biased in most species, resulting in male-male competition for access to females, and often choice of females among their possible mate. This general pattern differs however in numerous species (Trivers 1972, Clutton-Brock and Vincent 1991, Kvarnemo and Ahnesjö 1996), leading to a number of different mating systems.

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Biases in population sex ratios also have the potential to alter the direction of competition for a mate (Emlen and Oring 1977). One of the possible causes for sex ratio bias are genetic entities that induce an excess in the sex by which they are inherited (Hurst 1993, Werren and Beukeboom 1998). Some of these sex ratio distorters (SRD) are intracytoplasmic microorganisms that distort the sex ratio toward females, the sex allowing their transmission through the cytoplasm of the eggs (Hurst et al. 1997, Rigaud 1997). The microorganisms increase in prevalence in their host population by various mechanisms favouring infected females vs. uninfected ones (Hurst et al. 1997, Werren and O'Neill 1997). Consequently the population sex ratio evolves toward a female bias. SRD have the potential to reduce the proportion of males to such an extent as to change the OSR, and therefore to shift the direction of mating competition. A sex role reversal has recently been shown in the butterfly *Acraea encedon*, associated with the infection by male-killing bacteria (Jiggins et al. 2000). In populations where male-killing bacteria are at high prevalence, females form lekking aggregates because their reproduction is limited by access to males, while such swarms are absent when bacteria are less frequent. Most females were found not mated, confirming that a male deficiency could limit reproduction enough to induce severe population bottlenecks, as previously suggested (Juchault et al. 1993, Hatcher et al. 1999).

Terrestrial isopods (woodlice) are interesting biological models for studying the effect of SRD on the OSR. Most woodlice species harbour intracytoplasmic bacteria of the genus *Wolbachia* inducing reversion of genotypic males into functional females (Martin et al. 1973, Bouchon et al. 1998). Because of the maternal transmission of *Wolbachia*, infected mothers produce highly female-biased progenies. In the most intensively studied species, *Armadillidium vulgare*, prevalence of *Wolbachia* in the field population varies between 6% and 60%, and infected populations are always associated with an overall female-biased sex ratio (Juchault et al. 1993). These sex ratio biases are also found in other species infected by feminizing *Wolbachia* (Rigaud et al., 1997, 1999, Bouchon et al. 1998). *Wolbachia* infection therefore has potential to govern OSR in woodlice. However, woodlice have a long life cycle (several years), and generally only females provide parental care (embryos develop into an incubating pouch for several weeks) (see Warburg 1993 for a review). We might therefore expect a discrepancy between potential rate of reproduction, which could be higher in males than in females, and the female bias in sex ratio, for the overall achievement of the OSR. The purpose of the present study is to determine the "time in" of each sex in several species of woodlice infected by *Wolbachia* to check this assumption.

Materials and methods

Species studied and reproductive cycle of terrestrial isopods

The three studied species (*Armadillidium nasatum* [Budde-Lund], *Oniscus asellus* [Linné] and *Philoscia muscorum* [Scopoli]) were chosen because they belong to different families of Oniscidea (Crustacea, Isopoda; Vandel 1962), they harbour feminising *Wolbachia* (Bouchon et al. 1998), and they are easy to collect in large numbers in the wild.

Under temperate climates, Oniscidea females reproduce seasonally, between April and September. Females are potentially iteroparous and can produce multiple broods per year in favourable conditions (Warburg 1993). The onset and duration of female reproduction is mainly controlled by environmental factors, such as photoperiod or temperature (McQueen and Steel 1980, Mocquard et al. 1989), while male breeding is not influenced by photoperiod (Jassem et al. 1982). The reproductive cycle is narrowly associated with the female moulting cycle. In the sexual rest period, when oocyte maturation is blocked, females undergo growth moults. In terrestrial isopods, the exuviation of the body occurs in two phases, ecdysis of the posterior region occurring around 24 h before the anterior part. With the increase in photoperiod and temperature, ovarian maturation begins during a pre-parturial intermoult (PPI). In most species, this will occur even in absence of males (Mead 1976). The fertilisation is internal, the sperm being introduced in the female oviducts through two independent ventral genital apertures. The eggs are laid in a brood pouch (marsupium) consisting of the differentiation of lamellar structures (oostegites) covering the female's ventral face. The marsupium differentiates only during a special moult, known as parturial moult (PM). The embryos develop inside the marsupium and the young emerge about one month later. After the emergence of the young, according to species and environmental conditions, females can undergo another parturial moult or make a growth moult.

General rearing and breeding procedures

All the animals used during the laboratory experiments were sampled in the wild near Poitiers (Vienne, France, 46°40′ N) before the beginning of the reproductive period (February 98). Sample sites were at Mignaloux for *A. nasatum* and at Quinçay for *O. asellus* and *P. muscorum*. Immediately after sampling, animals were sexed, males and females separated, and some females dissected to verify that they had not been mated in the wild at this time (unmated females were needed for the experiments described below). Mated females are characterised by the presence of large white balls of sperm in their two genital ducts, while oviducts of non-mated females are thin and transparent. These animals were maintained on moistened soil at 20°C, under natural photoperiod. Food consisted of dead leaves and slices of fresh carrots and was provided ad libitum.

In all the following experiments, the animals were coupled with partners of approximately equal size, to avoid any possibility of physical incompatibility for reproduction. The pairs or groups of animals were placed in a cylindrical box (diameter 8 cm) with moistened soil and a piece of dead leaf, at 20°C under natural photoperiod during 24 h (time sufficient for mating to occur [Mead 1973]).

Female receptivity period and reproductive cycle

In most isopods, mating receptivity of females depends on the reproductive cycle stage: it can occur immediately before, during or after the parturial moult (Mead 1976, Warburg 1993). In a few species however, mating can occur at any time of each female intermoult (Mead 1976). To investigate female receptivity period in the three species studied here, 66 couples of *A. nasatum*, 56 couples of *O. asellus* and 35 couples of *P. muscorum* were made from April to June, i.e. when the photoperiod reached stimulating values (McQueen and Steel 1980, Mocquard et al. 1989). Animals were coupled, without a priori knowledge of the reproductive stage of females. The couple size (where size homogamy was respected) varied between 6.25 and 12.75 mm in *A. nasatum*, between 6.50 and 16.00 mm in *O. asellus*, and between 6.50 and 11.50 mm in *P. muscorum*. Females were dissected 24 h later, fertilisation status noted, and the diameter of ten oocytes measured under the microscope using an eyepiece micrometer. Female reproductive state was assessed from the stage of their ovarian development (Besse 1976). The bright, small (diameter < 250 µm) oocytes are those blocked in primary vitellogenesis. Dark, larger oocytes indicate a secondary vitellogenesis, which will be followed by egg laying at the following moult (parturial moult).

Relationship between mating and moulting in both sexes

To determine a possible moult cycle period during which females and males are not able to mate, test crosses were made in each species with animals at different stages of the moult cycle. They were coupled with a partner that was at intermoult stage, and fit for mating. Receptive females (as defined in the preceding experiment) undergoing posterior or anterior parturial ecdysis were tested. Receptive females with an empty marsupium following the release of young, were also tested. During this stage, the oostegites of the marsupium cover the genital apertures. Male were tested during the posterior and anterior ecdysis, and also during the stage immediately preceding ecdysis, when the old cuticle separates from the new one (a stage characterised by the presence of intense white plates of calcium carbonate on the ventral face; Steel and Campbell 1977). After 24 h, female mating status was checked as described above.

Potential mating capacity of males

To determine whether males are limited in the number of ejaculates they can deliver, five receptive females per day were provided to a single male over a period of four days. The five females were changed every 24 h, so that each male had 20 females available for mating.

Females were then dissected in order to examine their mating status and receptivity state, as previously described. Males that had been supplied with at least one non-receptive female were then excluded from the analysis. Four males of *P. muscorum*, five males of *A. nasatum* and seven males of *O. asellus* remained.

Field population analysis

The operational sex ratio might be modulated by several life history reproductive traits that could be assessed only in the wild (Kvarnemo and Ahnesjö 1996). With the aim of estimating the population sex ratio and the dynamics of the reproductive onset of females, samples were collected in the wild at the beginning of reproductive season (mid-April for *O. asellus* and *P. muscorum*; mid-May for *A. nasatum*). We have defined the onset of reproduction as the date at which the first receptive females were found (limited monthly samples were previously taken to determine this period). On each site, all visible animals under stones or leaf litter were gathered without size discrimination, on a surface of approximately 5 m^2. The woodlice were immediately sorted after collection, according to sex and stages of moulting cycle. The size (body length without antennae) of each individual was recorded using a binocular microscope with a graduated ocular. Around 40% of these females were sampled for dissection. This sample was representative of size and stages of moulting cycle of females collected in the wild. The dissection aimed to assess (1) the stage of female reproductive cycle, by measuring the diameter of ten oocytes; (2) the mating status, by checking presence or absence of sperm in oviducts; and (3) the prevalence of *Wolbachia*. For this, the nerve chord and one ovary of each female were taken for the extraction of total DNA as described by Kocher et al. (1989). Presence of *Wolbachia* infection was determined by PCR amplification of a part of the bacterial 16SrDNA, using *Wolbachia* specific primers, as described in Bouchon et al. (1998).

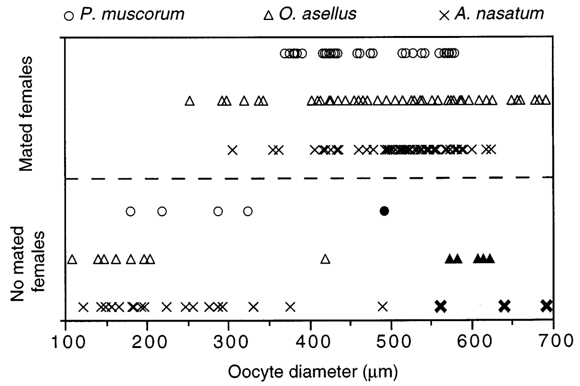

Fig. 1. Relationship between the maturation stage of oocytes and the mating status of females in three species of woodlice. Black symbols denote couples of animals with size < 7 mm (see text).

All statistical analyses were performed using the JMP (ver. 3.2.2) software (SAS Institute, Inc.).

Results

Female and male availability for mating

The dissection of females paired with a male for 24 h revealed that a minimal oocyte diameter must be reached before female mating (Fig. 1). This minimal diameter varied according to the species, but in all cases, the oocytes of mated females were dark yellow and > 250 µm in size, indicating accumulation of vitellus (Besse 1976). Females were therefore receptive to mating only after the beginning of secondary vitellogenesis, but this stage occurred at different oocyte diameters in different species. The oocyte maturation was therefore a necessary condition for females to be receptive to mating.

In all species, however, a few females were not mated while receptive (Fig. 1). Most of these females were paired with the smaller males: size below 7.0 mm for each species. We observed that the copulatory organs (the endopodites of the two first pairs of pleopods) of these males did not reach their maximal size, relative to other body parts. In addition, dissection of these small males revealed only a few sperm in their testes. The size of 7 mm could therefore be considered as the size at which males reached their sexual maturity. Their mates were receptive to mating (Fig. 1), suggesting that females are potentially mature before males. A few receptive females coupled with mature males (size > 10 mm) were not mated (1.8% for *O. asellus*, 4.5% for *A. nasatum*), suggesting that some males were not available to mating or were not attracted by the females. These males were dissected, and appear to have sperm reserves and were not parasitised.

Relationship between mating and moulting in both sexes

For all species, the mating availability varied according to the stage of the moulting cycle (Table 1). In males, mating was possible even a few hours before ecdysis, when the old cuticle began to separate from the new one, but was not possible during the whole ecdysis period (posterior and anterior moult). The same result was obtained with the females. In addition, in most cases, receptive females with an empty marsupium (after the release of young) were not able to breed (Table 1). The exception was *A. nasatum*, where 41.7% females were able to be mated even when oostegites masked the genital apertures.

Potential mating capacity of males

Males of each species were able to mate with a large number of females during the whole trial run (7.6 ± 1.2 females mated for *A. nasatum*, 6.4 ± 1.0 for *O. asellus* and 7.2 ± 1.3 for *P. muscorum*). An analysis of covariance, using the day of the experiment as covariate, showed that these values were not significantly different between species ($F_{2,52} = 0.53$, $P > 0.55$). The day of the experiment had a strong effect on the number of females mated ($F_{3,52} = 24.6$, $P < 0.0001$): for all species, the number of females inseminated by the male decreased with the day (Fig. 2). The interaction between the species and the day of the experiment was also significant ($F_{6,52} = 3.27$, $P < 0.009$). Different mating strategies were therefore used between species over time (Fig. 2). In *P. muscorum*, males have mated the whole

Table 1. Mating availability in relation to the different steps of the moult cycle in three terrestrial isopods.

Species	Males			Females		
	EIM	PM	AM	PPM	APM	Marsupium
A. nasatum	10 (10)	0 (10)	0 (10)	0 (10)	0 (10)	5 (12)
O. asellus	10 (11)	0 (10)	0 (10)	0 (10)	0 (10)	0 (30)
P. muscorum	–	–	–	0 (4)	0 (6)	0 (13)

EIM: end of the intermoult, stage where the old cuticle separates from the new one; PM: posterior moult; AM: anterior moult; PPM: posterior parturial moult; APM: anterior parturial moult. The numbers of tested individuals are indicated within parenthesis. "–" denotes that no experiment was done (no individuals available at that stage of the moult cycle).

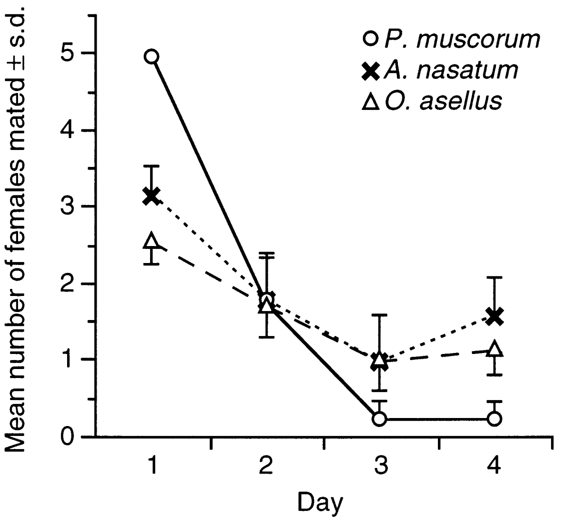

Fig. 2. Mean number of woodlice females mated per male, during four days. Five receptive females were proposed to each male every day.

female set the first day and nearly none during days 3 and 4. This strategy contrasts with the other species that mated fewer females at the beginning, but continued mating every day.

Field population analysis

In each species, female-biased sex ratios were observed. The bias was not significant from a 1:1 sex ratio in *A. nasatum* ($\chi_1^2 = 1.58$, $P > 0.20$), but was highly significant in the other species ($\chi_1^2 = 17.8$, $P < 0.0001$ in *O. asellus*; $\chi_1^2 = 12.3$, $P < 0.0006$ in *P. muscorum*) (Table 2). Correlation between species revealed no direct link between *Wolbachia* prevalence and sex ratio bias. This could be due to an artefact of small sample sizes used for the estimation of symbiont prevalence (Table 2).

Only between 27% and 52% females were receptive to mating (i.e. in "time in") at the beginning of the reproductive season (Table 2). Even females already mated were counted in "time in", since previous studies have shown, directly or indirectly, that females can receive several matings during a single intermoult (Lueken 1966, Sassaman 1978). In each species, the bigger females were receptive before the smaller ones (Table 2). Given the relationship between size and age in woodlice, this means that older females are more sexually precocious than younger ones. There was no difference in *Wolbachia* infection between receptive or non-receptive females (Table 3; Fisher exact test N.S. for each species). At the same time, most of the males were able to mate (Table 2), with the exception of *A. nasatum*, for which a high number of young males (≤ 6 mm) were found. The operational sex ratios (OSR) were estimated from the overall population sex ratio

Table 2. Sex ratio, *Wolbachia* prevalence and proportion of individuals in reproductive "time in" and "time out", in natural populations of three woodlice species collected at the beginning of the reproductive period.

Species		Males		Females						
	N	*n*	*n*Ti	*n*[a]	% Wo (C.I. limits)	*n*To (mean size ± sd)	*n*Ti (mean size ± sd)	*P*[b]	PSR (C.I. limits)	OSR (C.I. limits)
A. nasatum	103	43	25	22	50.0 (28.5–71.1)	16 (7.6 ± 2.3)	6 (11.7 ± 1.0)	<0.001	41.7 % (33.7–49.7)	60.9 % (48.4–73.4)
O. asellus	139	35	28	43	32.6 (19.0–49.0)	19 (7.6 ± 1.3)	24 (11.9 ± 1.3)	<0.0001	25.2 % (19.2–31.2)	33.9 % (25.5–42.4)
P. muscorum	92	23	23	29	82.7 (64.9–94.1)	20 (8.5 ± 0.7)	9 (10.0 ± 0.6)	<0.0002	25.0 % (17.6–32.4)	51.8 % (40.6–63.0)

*n*Ti: number of individuals in "time in", i.e. able to mate at the date of sampling; *n*To: number of individuals in "time out", i.e. unable to mate at that time (see laboratory experiments for defining Ti and To for each sex); %Wo: proportion of females habouring *Wolbachia* endosymbionts; C.I.: binomial confidence interval limits; PSR: population sex ratio = $n♂/N$; OSR: operational sex ratio = $(\%♂Ti \times n♂)/(\%♂Ti \times n♂ + \%♀Ti \times n♀)$. The size of females is given in mm. [a]number of females tested; [b]Wilcoxon rank test on female size.

Table 3. *Wolbachia* frequencies in females according to their reproductive stage.

Species	NR, not mated		R, mated		R, not mated	
	Wo+	Wo−	Wo+	Wo−	Wo+	Wo−
A. nasatum	7	9	1	2	3	0
O. asellus	7	12	5	16	2	1
P. muscorum	17	3	3	2	4	0

R: females receptive to mating at the date of sampling; NR: females not receptive to mating at that time; Wo+: females infected by *Wolbachia* (positive PCR test); Wo−: females uninfected by *Wolbachia* (negative PCR test).

and the proportion of "time in" of each sex (Table 2). OSR were more or less balanced for *A. nasatum* and *P. muscorum* but still female-biased for *O. asellus*. These OSR always included a higher proportion of males than predicted by looking at the overall population sex ratio only (Table 2).

Some receptive females were found not mated in the wild (Table 3). A logistic regression testing both the effects of species and *Wolbachia* infection on mating status revealed no significant effect of the species, but a significant effect of *Wolbachia* infection: the proportion of infected females was higher among the non-mated females than among the mated ones (whole model: $\chi^2_3 = 13.03$, $P < 0.005$; effect of the species: Wald $\chi^2_2 = 1.59$, $P > 0.45$; effect of the infection: Wald $\chi^2_1 = 5.24$, $P < 0.03$).

Discussion

The notable feature brought out by our study is that the operational sex ratio (OSR) in the three woodlice species is determined by the balance of two opposite forces: (1) the high potential rate of reproduction in males and asynchrony in female onset of reproduction, which bias the OSR toward males and (2) female-biased sex ratios induced by feminising cytoplasmic symbionts.

Female mating receptivity is limited to a period preceding the laying, i.e. during the final stage of oocyte maturation. The duration of this period varies between 15 and 60 d (generally around 30 d), depending on environmental conditions (Steel 1982, Caubet et al., 1998). Furthermore, females are unable to mate when carrying eggs (the eggs prevent access to genital apertures, and females are not receptive at this moment; Besse 1976), and often between two successive broods, because of the presence of the marsupium. A few females of *Armadillidium nasatum* can nevertheless be mated at that time, confirming the results obtained in the closely related species *Armadillidium vulgare* (Mead 1973). However, the female "time in" period of terrestrial isopod species studied here is generally limited compared to their potential life time (between one and three years, Warburg 1993). On the other hand, most of the adult males are able to breed at any time, except during the short period of ecdysis, (usually 2–3 d per month according to our observations). This is true at least in thermal conditions compatible with the physiological activity (Jassem et al. 1982), which is the case during the reproductive season in the wild. Furthermore, male mating capacity was high on a relatively short time scale, so that a small number of males can potentially mate with a large number of females. However, a few adult males did not mate in our experiments when they were coupled with receptive females. This could be due to variability in the male breeding success, but also to the fact that males are less likely to breed with females infected by *Wolbachia* (see below). But the infection status of females used in our laboratory experiments was unknown, so the cause for the lack of mating in these experiments remains unknown. Globally, however, the potential reproductive rates (PRR) in males were much higher than in females, a classic situation for species where females invest more in reproduction than males (Trivers 1972). This difference in PRR would predict that males will have much more opportunity to breed than females during their life cycle, leading to a male-biased OSR in populations (Kvarnemo and Ahnesjö 1996).

This tendency to male-biased OSR is reinforced by the fact that, in the three species, females were asynchronous for their onset of reproduction, which decreases the proportion of females receptive for mating at any time.

Our observations in the wild tend to show that population sex ratios are generally female-biased, confirming previous data in terrestrial isopods (e.g. Juchault et al. 1992, Rigaud et al., 1997, 1999). The *Wolbachia* harboured by the three species studied here are responsible for the female bias in primary sex ratios (Juchault and Legrand 1979, Bouchon et al. 1998, Rigaud et al. 1999), hence these endosymbionts are likely to be a primary cause for the population-biased sex ratio. The three populations studied here were, however, polymorphic for the *Wolbachia* infection, and the male rarity was not extreme. The lack of a strict correlation between *Wolbachia* prevalence and population sex ratio between species could indicate either sample bias or other parameters influencing the sex ratio: differences between species in survival between sexes, or differences in the efficiency of *Wolbachia* feminisation (as suggested by Rigaud et al. 1999). On average, however, everything else being equal, an excess of females would allow the OSR to be female-biased at the beginning of the reproductive season.

The combination of these three phenomena (higher PRR in males, asynchronous reproductive onset in females, female-biased sex ratios) leads to a more or less balanced OSR (or slightly female-biased depending on the species) at the beginning of the reproductive season. Because of reproduction asynchrony, this balance would vary only slightly until the moment where all females have undergone their parturial moults. The low proportion of males observed in two species in the present study, and which can reach 19% males in other species such as *Armadillidium vulgare* (Juchault et al. 1992), could therefore not be seen as a key factor for population extinction or limitation, as suggested by Juchault et al. (1993) or Hatcher et al. (1999). The OSR values estimated in this study must nevertheless be used with caution: our lab experiments showed that males suffer differences in their mating capacity after several matings. Our experimental procedure did not allow us to assess if males captured in the wild suffer such differences. In addition, as females can be inseminated more than once during their receptive period (see below), the high PRR of males could be necessary to inseminate a large number of "in demand" females (if females are "in demand" of several inseminations). The OSR could therefore be more female-biased than estimated. However, more data are needed to assess the females' need for multiple matings. Also, the number of sperm in male ejaculates declines after multiple mating (Moreau unpubl.), a phenomenon that could decrease male fertility, and therefore overestimate the PRR of males and induce more female bias in the OSR.

Some receptive females were found not mated in the wild. These non-mated females were more often infected by *Wolbachia*. This could be due to at least two phenomena. A first explanation could be that our samples consisted of many patches, with differences in *Wolbachia* prevalence among the patches. Females in patches with high *Wolbachia* prevalence, therefore including very few males, would tend to be less mated. The combination of all patches could then lead to the overall observed tendency. More data on the microstructure of populations are needed to test this possibility. Alternatively, the males could selectively avoid infected females as first mates. This could be expected if females suffer a loss of fitness due to the *Wolbachia* infection, and if males are able to distinguish between infected and uninfected females. The fecundity decrease associated with the infection in *O. asellus* (Rigaud et al. 1999) could explain why males could avoid infected females as mates, and the existence of a behavioural courtship before mating in woodlice (Mead 1973) suggests that males are capable of assessing their mate. Again, more data are needed to test this hypothesis.

In the butterfly *Acraea encedon*, the sex ratio distorting *Wolbachia* has a very important impact on population dynamics and mating system of the host, mainly because the sex ratio bias is very high and the life span very short in this species. Most of the females probably never mate in the most female-biased populations (Owen et al. 1973, Jiggins et al. 2000), but a higher probability was found for uninfected females to be mated, which should induce a frequency-dependent dynamic of the *Wolbachia* prevalence in populations (Jiggins et al. 2000). Such a tendency was found in woodlice, but the phenomenon is weaker because males have a strong mating capacity and females can delay their parturial moult in the absence of males (Lefebvre and Caubet 1999), so that unmated females observed at a given moment could probably be mated later (this point needs verification, however). But mating with infected females as a "second choice" could at least have consequences on the number of matings they can experience. Within a given cohort of receptive females, if males favour mating with one given type of females, those chosen as secondary mates would have less possibilities for multiple mating because male mating capacity decreases after several matings in a short time (see Fig. 2). Earlier studies showed that woodlice females can be mated several times during a single preparturial intermoult (Lueken 1966, Sassaman 1978), and that young of nearly 80% of females are issued from sperm of several males in natural conditions (Sassaman 1978). The high frequency of multiple mating in woodlice therefore suggests that it provides an advantage to females (whatever this advantage, see Krebs and Davies 1997, Hosken and Blanckenhorn 1999), an advantage that uninfected females would be more likely to experience.

Our study raises a last problem. It is possible that the asynchronous onset of female reproduction might be the result of a selection due to female-biased sex ratios imposed by *Wolbachia* allowing more females to be mated. This asynchrony could also be due to a plasticity, i.e. a proximate response of individuals to local sex ratio bias. Only studies in the wild including numerous sample sites per species, with variation in *Wolbachia* prevalence, would allow one to choose among these hypotheses. Such a study would also allow one to compare OSR and females' unmatedness across populations with different *Wolbachia* prevalence.

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